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Increase in Blood–Brain Barrier Permeability, Oxidative Stress, and Activated Microglia in a Rat Model of Blast-Induced Traumatic Brain Injury

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Traumatic brain injury (TBI) as a consequence of exposure to blast is increasingly prevalent in military populations, with the underlying pathophysiological mechanisms mostly unknown. In the present study, we utilized an air-driven shock tube to investigate the effects of blast exposure (120 kPa) on rat brains. Immediately following exposure to blast, neurological function was reduced. BBB permeability was measured using IgG antibody and evaluating its immunoreactivity in the brain. At 3 and 24 hr postexposure, there was a transient significant increase in IgG staining in the cortex. At 3 days postexposure, IgG immunoreactivity returned to control levels. Quantitative immunostaining was employed to determine the temporal course of brain oxidative stress following exposure to blast. Levels of 4-hydroxynonenal (4-HNE) and 3-nitrotyrosine (3-NT) were significantly increased at 3 hr postexposure and returned to control levels at 24 hr postexposure. The response of microglia to blast exposure was determined by autoradiographic localization of ^{3}H-PK11195 binding. At 5 days postexposure, increased binding was observed in the contralateral and ipsilateral dentate gyrus. These regions also displayed increased binding at 10 days postexposure; in addition to these regions there was increased binding in the contralateral ventral hippocampus and substantia nigra at this time point. By using antibodies against CD11b/c, microglia morphology characteristic of activated microglia was observed in the hippocampus and substantia nigra of animals exposed to blast. These results indicate that BBB breakdown, oxidative stress, and microglia activation likely play a role in the neuropathology associated with TBI as a result of blast exposure.

Key words: traumatic brain injury; oxidative stress; inflammation; blood–brain barrier; PK11195

Accumulating clinical evidence as well as experience in contemporary military operations suggests that substantial short-term and long-term neurologic deficits can be caused by blast exposure without a direct blow to the head (Trudeau et al., 1998; Cernak et al., 1999; DePalma et al., 2005; Ling et al., 2009; Elder and Cristian, 2009). With an estimated 15% of troops serving in Iraq sustaining some level of neurological impairment resulting from blast exposure, TBI has become the signature injury of this war (Hoge et al., 2008). Hoge and colleagues reported a high correlation between service members who reported symptoms consistent with mild TBI and those who received exposure to blast. Although sharing common clinical features with both, brain injury caused by blast (bTBI) is clinically distinct from closed-head and/or penetrating TBI (Ling et al., 2009). Although the exact biophysics underlying bTBI are not completely understood, interaction with a fast-moving, transient pressure wave is generally accepted as the primary cause of brain injury that results from blast exposure (Elsayed, 1997; DePalma et al., 2005; Taber et al., 2006). Experimental studies in rodents have demonstrated a correlation between memory dysfunction and distribution of neuropathological damage in the brain after exposure to blast overpressure (BOP; Cernak et al., 2001a,b).

Previous studies have also demonstrated that rats exposed to low- and moderate-intensity BOP (83 kPa or 112 kPa) had lowered food intake and exercise performance (Bauman et al., 1997), with similar findings

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reported in sheep (Mundie et al., 2000). Rats exposed to low-intensity BOP (20 kPa) displayed significant performance deficits on rotametric and grip-strength tests (Moochhala et al., 2004; Saljo et al., 2009), whereas animal studies using moderate levels of BOP (126 kPa) have shown Morris water maze performance impairment, gliosis, and fiber degeneration (Long et al., 2009). Reactive gliosis, neuronal swelling, and cytoplasmic vacuolation have also been observed in the hippocampus of rats subjected to thoracic blast injury (Cernak et al., 2001a,b). Rhesus monkeys exposed to high BOP (207 kPa, 276 kPa, or 345 kPa) demonstrated significant, albeit transient, memory and performance deficits (Bogo et al., 1971).

TBI can result in brain microvasculature and blood–brain barrier (BBB) breakdown, leading to increased BBB permeability. Disruption of the BBB following TBI results in brain edema, a primary event that affects both morbidity and mortality following TBI (Unterberg et al., 2004). Edema increases intracerebral pressure (ICP) and leads to secondary ischemic injuries by impairing cerebral perfusion and oxygenation (Unterberg et al., 2004). After TBI, various mediators are released that enhance vasogenic and/or cytotoxic brain edema. These include glutamate, lactate, H^+, K^+, Ca^{2+}, nitric oxide, arachidonic acid and its metabolites, free oxygen radicals, histamine, and kinins (Unterberg et al., 2004). BBB permeability to endogenous proteins, such as immunoglobulins (IgG), is increased following experimental TBI (Tanno et al., 1992; Sullivan et al., 2000). An additional consequence of BBB disruption is the infiltration of leukocytes into brain tissue, activation of microglia, and inflammation (Morganti-Kossmann et al., 2007).There is some indication of a widespread activation of microglia 1–14 days after exposure to blast; however, it remains unclear whether this activation was a direct result of blast wave or was caused indirectly by an increase in permeability in the BBB and transfer of inflammatory mediators from circulation (Kaur et al., 1995). Oxidative stress has been shown to play a critical role in the secondary injury process following TBI. In recent work, our colleagues demonstrated that, in both controlled cortical impact and weight drop models of TBI, there is a rapid increase in the lipid peroxidation marker 4-hydroxynonenal (4-HNE) and the protein nitration marker 3-nitrotyrosine (3-NT; <30 min postinjury), which returned to control levels 24 hr postinjury (Hall et al., 2004; Deng et al., 2007).

PK11195, an isoquinoline derivative, is an antagonist of the translocator protein (TPSO; 18 kDa; Banati et al., 1997). TPSOs, previously referred to as the *peripheral benzodiazepine receptor* (PBR), play an important role in steriodogenesis by acting as cholesterol pores in the outer mitochondrial membrane (Scarf et al., 2009). TPSOs are absent from neurons but are expressed at high density in glial and ependymal cells (Chen and Guilarte, 2008). Increased PK11195 binding has been localized to activated microglia in several animal models of CNS injury, including TBI, ischemia, and excitotoxic lesion models (Dubois et al., 1988; Gotti et al., 1990; Grossman et al., 2003). The use of TPSO autoradiographic analysis as a marker of inflammation is advantageous over other methods because of the high sensitivity and anatomical resolution of the technique (Benavides et al., 2001).

To begin to elucidate the mechanism(s) involved in bTBI neuropathology, the current study was designed to assess acute changes in neurological function and to characterize acute changes in BBB permeability and oxidative damage and the subacute inflammatory response of the brain following BOP exposure. The results demonstrate the novel findings that after exposure to a moderate BOP (120 kPa) there is significant reflex suppression, disruption of the BBB, oxidative stress, and widespread microglia activation. Taken together, these data suggest that bTBI shares mechanisms of pathobiology that are hallmarks of injury in other models of TBI, which may widen future avenues of neuroprotective interventions for blast injuries.

MATERIALS AND METHODS

Animals and Exposure to Blast

Adult, virus-free male Sprague-Dawley rats (250–300 g; n = 49) were randomly divided into blast-exposed (n = 44) and control (n = 14) groups. Animals were anesthetized with isoflurane and positioned in a holder that prevented secondary and tertiary blast injuries as previously described (Chavko et al., 2006). Animals were then placed into the end of the expansion chamber of an air-driven shock tube (2.5 ft compression chamber connected to a 15 ft expansion chamber) with their right side ipsilateral to the direction of the BOP. Animals assigned to exposures were subjected to a peak overpressure of 120 kPa. Control animals received anesthesia and were treated in the same way except for the exposure to BOP.

Acute Neurological Assessment

Acute neurological function was assessed by using a battery of tests that are analogous to motor components of the Glasgow Coma Scale (GCS) as previously described (Dixon et al., 1987). These tests measure the duration of suppression of a response due to injury. Immediately following BOP exposure or control treatment, the duration of suppression of three reflexes were acutely evaluated. The reflexes of control animals were evaluated in order to determine the effects of anesthesia on reflex suppression. The corneal reflex was assessed by lightly touching the eye to elicit a blinking response. The paw flexion reflex was assessed by briefly applying a pinch (approximately 0.2 kg/mm^2) to the hind paw to elicit a withdrawal response. The righting response was tested by placing the animal on its back and timing the return to a spontaneous upright position. Evaluation continued until all reflexes had returned.

Immunohistochemistry

For IgG immunohistochemical assessment, 24 animals were exposed to blast and allowed to recover for 0.5, 3, 24,

or 72 hr following BOP exposure (n = 6/group). In total six control animals were handled in a similar manner and did not receive any BOP exposure. At the designated survival intervals, animals were anesthetized with pentobarbital (95 mg/kg body weight) and transcardially perfused with phosphate-buffered saline (PBS), followed by 4% paraformaldehyde, pH 7.4. After removal, the brains were placed in 4% paraformaldehyde-sucrose (15%) for an additional 24 hr. Coronal sections (50 μm) were then cut using a freezing microtome throughout the rostral caudal extent of the brain, extending through the septal area to the most posterior extent of the hippocampus. BBB permeability was assayed on every twelfth section from each animal with biotinylated anti-rat IgG antibody as previously described (Hoane et al., 2006). Sections were rinsed with PBS and incubated in 0.3% H_2O_2 in order to block endogenous peroxidase activity. Next, sections were blocked in 10% normal goat serum with 0.2% Triton X-100 in PBS for 1 hr at room temperature, followed by incubation in primary antibody overnight at 4°C (1:1,000; biotinylated goat anti-rat IgG; Vector, Burlingame, CA). Sections were rinsed and incubated in avidin-horseradish peroxidase complex (Vectastain ABC) for 1 hr. IgG immunoreactivity was visualized by incubation of sections in 0.05% diaminobenzidine, 0.01% H_2O_2, and 0.3% imidazole for 10 min. Finally, sections were rinsed with PBS, mounted on gelatinized slides, and coverslipped with permount. IgG imunoreactivity was quantified b y using a gray-level-index (GLI) as previously described (Bisler et al., 2002). Quantification was performed in Image-Pro Plus software (Media Cybernetics, Bethesda, MD) such that GLI values corresponded to the area of immunoreactivity in relation to an area of interest. The GLI value for each section (~12 sections per animal) were calculated and averaged in order to determine the percentage of total brain area immunoreactive for IgG. These values were then averaged for each group.

For immunohistochemical detection of brain oxidative stress levels, six animals were subjected to BOP and allowed to recover for either 3 hr or 24 hr following BOP exposure. Three control animals were handled in a similar manner and did not receive any BOP exposure. At the designated survival intervals, animals were anesthetized with pentobarbital and perfused with PBS and 4% paraformaldehyde. Brains were placed in 4% paraformaldehyde-sucrose (15%) for an additional 24 hr. Coronal sections (50 μm) were then cut using a freezing microtome throughout the rostral caudal extent of the brain, extending through the septal area to the most posterior extent of the hippocampus. Free-floating sections were double labeled for 3-NT (1:1,000 mouse anti-3-NT monoclonal antibody; Upstate, Lake Placid, NY) and 4-HNE (1:5,000 rabbit anti-HNE polyclonal antibody; Calbiochem, San Diego, CA) and visualized by infrared immunohistochemistry as previously described, with a few modifications (Hawes et al., 2005). Briefly, sections were washed three times for 5 min each in PBS, followed by adduct reduction in 0.1 M NaBH4 in 0.1 MOPS (Sigma, St. Louis, MO) for 10 min. After reduction, sections were rinsed three times for 5 min each in PBS, followed by blocking in 10% normal goat serum with 0.2% Triton X-100 in PBS for 1 hr at room temperature. Sections were incubated in primary antibodies overnight at 4°C. Before incubation in secondary antibody, sections were washed three times for 5 min each in PBS. Sections were then incubated with secondary antibodies for 2 hr at room temperature (1:20,000 IRDye700DX-conjugated anti-mouse IgG; 1:10,000 IRDye800;conjugated anti-rabbit IgG). All sections were rinsed three times for 5 min each in PBS and mounted onto Fisher Superfrost Plus slides. Slides were scanned simultaneously on a LI-COR Odyssey Infrared Imager (LI-COR Biosciences). The fluorescence intensity for each section (~12 sections per animal) was quantified using an area of similar size throughout each section. To obtain a value for each animal, the levels of 3-NT and 4-HNE for each section were averaged. These values were then averaged for each group.

For assessment of microglia morphology, antibodies against CD11b/c that recognize microglia-specific complement type 3 receptor were utilized. Briefly, endogenous peroxidase activity was blocked using 0.3% H_2O_2. Next, sections were blocked in 10% normal goat serum with 0.2% Triton X-100 in PBS for 1 hr at room temperature, followed by incubation in primary antibody overnight at 4°C (1:1,000 mouse anti-rat CD11b/c; BD Pharmigen, San Diego, CA). Sections were then incubated in secondary antibody for 2 hr at room temperature (1:500; biotinylated goat anti-mouse; Vector). Sections were rinsed and incubated in avidin-horseradish peroxidase complex (Vectastain ABC) for 1 hr. IgG immunoreactivity was visualized by incubation of sections in 0.05% diaminobenzidine, 0.01% H_2O_2, and 0.3% imidazole for 10 min. Finally, sections were rinsed with PBS, mounted on gelatinized slides, and coverslipped with Permount.

[^{3}H]PK11195 Autoradiography

For autoradiography studies, 14 animals were subjected to BOP and allowed to recover for either 5 or 10 days following exposure (n = 7/group). Five control animals were given the same treatment except for BOP exposure. Animals were euthanized, and their brains were immediately removed and frozen in isopentane on dry ice. Brains were sectioned (16 μm) on a cryostat and mounted on Fisher Superfrost Plus slides. TPSO autoradiography was performed using 1 nM [^{3}H]PK11195 ligand (PerkinElmer, Boston, MA; specific activity 85.5Ci/mmol) as previously described (Kelso et al., 2009). Brain sections were preincubated in 50 mM Tris-HCl, pH 7.4, at 4°C for 15 min, followed by incubation with radioactive ligand at 4°C for 2 hr. The binding was terminated by washing sections in 50 mM Tris-HCl, pH 7.4 (3 × 3 min), followed by dipping in ice cold water. The sections were exposed to Kodax BioMax Autoradiography Film (Kodak, Rochester, NY) for 55 days. All films were developed using a Kodak D-19 developer and analyzed in NIH Image v1.59 on a Power Macintosh connected to a Sony XC-77 CCD camera via a Scion LG-3 frame-grabber (Scion, Inc., Frederick, MD). Autoradiograph data was quantified by densitometry.

Statistical Analysis

Individual reflexes (corneal, paw flexion, and righting) were analyzed by one-tailed *t*-test. Data from immunohistochemical assays were analyzed by one-way ANOVA followed

by Student Newman-Keuls (SNK) post hoc analysis. [^{3}H]PK11195 autoradiography results were analyzed by two-way repeated measures (hemisphere) ANOVA followed by Bonferroni post hoc analysis. Significance was accepted at $P < 0.05$. All values are presented as means ± SEM. The experiments reported herein were conducted according to the principles set forth in the *Guide for the care and use of laboratory animals* (Institute of Laboratory Animal Resources, National Research Council, National Academy Press, 1996) and were approved by the WRAIR/NMRC IACUC Committee.

RESULTS

Effect of Blast Overpressure on Acute Neurological Function and Brain Morphology

Recently, Hamm (2001) demonstrated that reflex suppression is a sensitive predictor of injury effect; therefore, acute neurological deficits as a result of BOP exposure were assessed using a battery of reflexes that test somatomotor function. The survival rate of animals following BOP exposure was 98%. The return of the corneal reflex in BOP-exposed animals (23.6 ± 2.2 sec) was significantly delayed compared with control animals (14.33 ± 6.6 sec; $P < 0.05$). Additionally, BOP exposure resulted in a significant suppression of the paw flexion reflex (47.7 ± 4.0 sec) compared with control animals (21.8 ± 4.5 sec; $P < 0.01$; Fig. 1). However, no significant differences were measured between the righting reflexes of the two groups. There was no evidence of tissue disruption or tissue loss in BOP-exposed animals (Fig. 2).

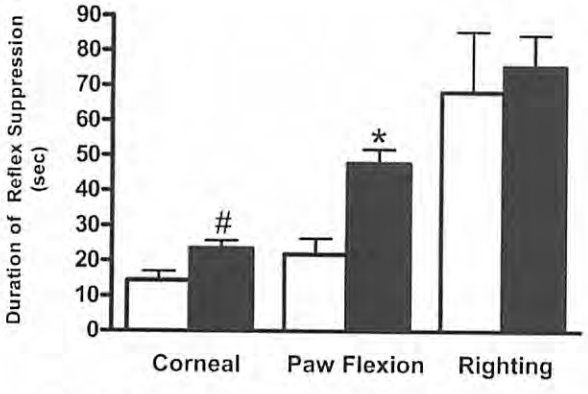

Fig. 1. Effect of BOP on acute neurological functioning immediately following exposure. Exposure to blast (solid columns) significantly delayed the return of corneal and paw flexion reflexes compared with control (open columns). The data represent the mean duration of suppression ± SEM. Significance (*t*-test) is denoted as follows: $^{\#}P < 0.05$, $^{*}P < 0.01$ vs. control.

Effect of BOP on IgG Immunoreactivity

The spatial extravasation of endogenous IgG immunoreactivity was used as an index for BBB breakdown following BOP exposure. BBB disruption is a well-established hallmark following brain injury and has been assessed in several models of TBI. Qualitative assessment of IgG extravasation revealed that at 3 and 24 hr following BOP exposure there was a conspicuous increase in IgG immunoreactivity in the outer most layer of the cortex. An ANOVA revealed a significant group difference ($F[4,22] = 12.77$, $P < 0.0001$) in IgG immunoreactivity following BOP exposure. After BOP exposure, post hoc comparisons revealed a significant increase in IgG immunoreactivity at 3 hr ($P < 0.001$) and 24 hr ($P < 0.01$) post-BOP exposure (Figs. 3, 4). Although it was not significant, there was a trend toward increased IgG immunoreactivity at 0.5 hr postinjury surrounding the lateral ventricles.

Effect of BOP on Oxidative Stress

Oxidative stress is known to play a role in the secondary injury cascade following TBI; in the present study, we determined 3-NT and 4-HNE levels following BOP exposure using quantitative immunohistochemistry. An ANOVA revealed a significant group difference ($F[2,6] = 11.24$, $P < 0.01$) in 4-HNE levels following BOP exposure at 3 hr postexposure ($P < 0.01$). 4-HNE levels returned to control values at 24 hr postexposure. Similarly, there was a significant group difference ($F[2,6] = 11.55$, $P < 0.01$) in 3-NT levels at 3 hr following

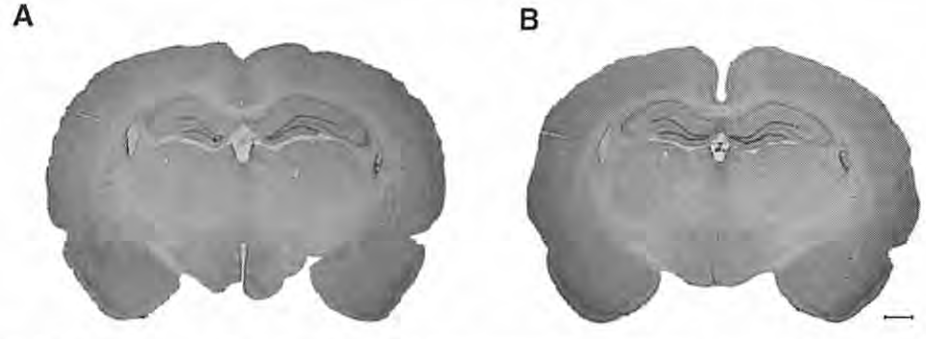

Fig. 2. Representative images of H&E-stained brain sections from controls (**A**) and at 3 days postblast 120 kPa (**B**). There were no overt signs of tissue disruption or cell loss. Scale bar = 1 mm.

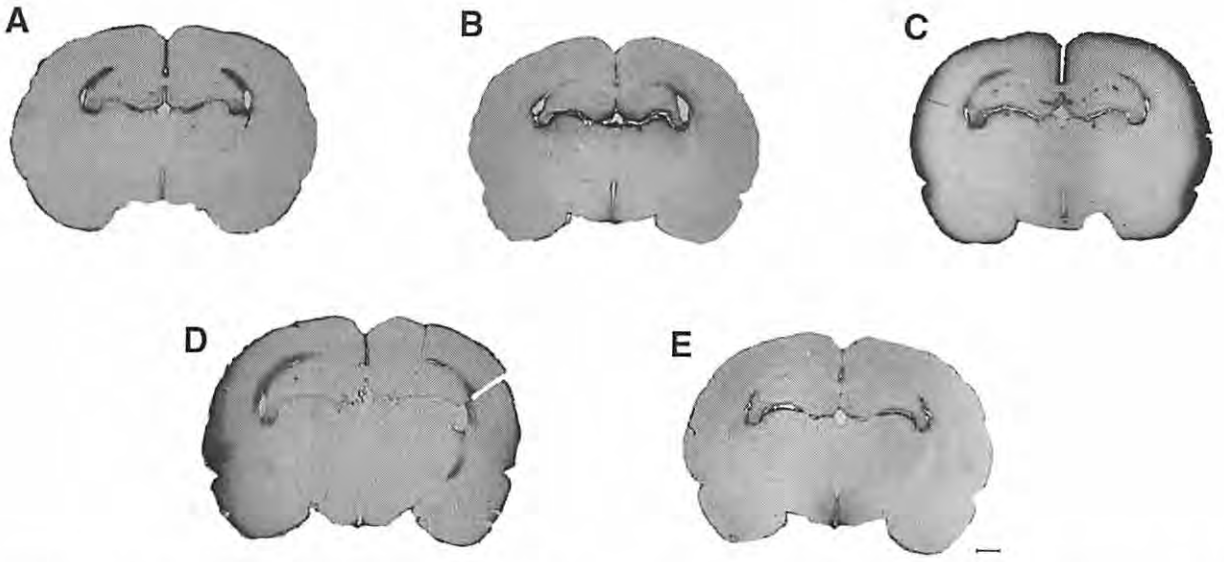

Fig. 3. Effect of 120 kPa BOP on brain IgG immunoreactivity. The images are representative for control animals (**A**), 0.5 hr postblast (**B**), 3 hr postblast (**C**), 24 hr postblast (**D**), and 3 days postblast (**E**). Increased IgG immunoreactivity in layer I of the cortex was observed at 3 and 24 hr following exposure. Although it was not significant, there was increased staining in the area surrounding the lateral ventricles (four of six brains) at 0.5 hr postblast. There was no difference in IgG staining in brains 3 days postexposure compared with controls. Scale bar = 1 mm.

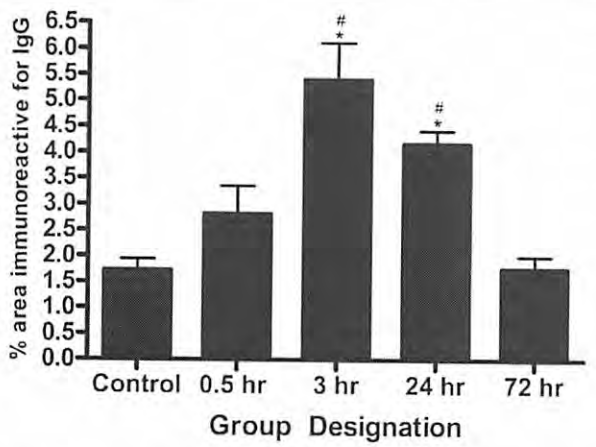

Fig. 4. Quantification of IgG immunoreactivity in brain sections after exposure to blast. Significant increases in IgG staining were observed at 3 and 24 hr after exposure compared with control. The data represent the percentage of brain area stained for IgG ± SEM. Significance with ANOVA followed by post hoc analysis (SNK) as follows: $^{*}P < 0.01$ vs. control; $^{\#}P < 0.05$ vs. 72 hr.

exposure ($P < 0.05$), which returned to control values at 24 hr postexposure (Fig. 5).

Effect of BOP on TPSO Binding and Microglia Morphology

Inflammation has been demonstrated in multiple models of TBI; in the present study, microglial activation was determined using TPSO autoradiographic localization. The effects of blast exposure on TPSO density were evaluated using [^{3}H]PK11195 autoradiography in several brain regions at 5 and 10 days postblast (Fig. 6). A two-way ANOVA was significant for effect of BOP exposure ($F[2,16] = 2.287$, $P < 0.001$) but not for hemisphere ($F[1,16] = 11.20$, $P > 0.05$; Table I). No significant interaction between BOP exposure and hemisphere existed ($F[2,16] = 1.494$, $P > 0.05$). At 5 days postblast, post hoc testing revealed a significant increase in the density of [^{3}H]PK11195 binding in the ipsilateral dentate gyrus ($P < 0.001$) and contralateral dentate gyrus ($P < 0.05$). At 10 days postblast, post hoc comparisons revealed a significant increase in [^{3}H]PK11195 binding in the contralateral dentate gyrus ($P < 0.001$), ipsilateral dentate gyrus ($P < 0.001$), contralateral substantia nigra ($P < 0.01$), and contralateral ventral hippocampus ($P < 0.05$).

To demonstrate further the response of microglia to BOP exposure, antibodies against CD11b/c were used to visualize microglia morphology. In the cortex, hippocampus, and substantia nigra of control animals, microglia demonstrated a resting morphology characterized by highly ramified processes (Fig. 7). In contrast, microglia in the hippocampus from animals exposed to blast displayed an activated morphology characterized by clustering. In addition to this, microglia in the substantia nigra of BOP-exposed animals revealed activated morphology characterized by rounded cell bodies and fewer ramifications.

DISCUSSION

Brain injury in rats as a result of exposure to BOP using an air-driven shock tube was used to study the

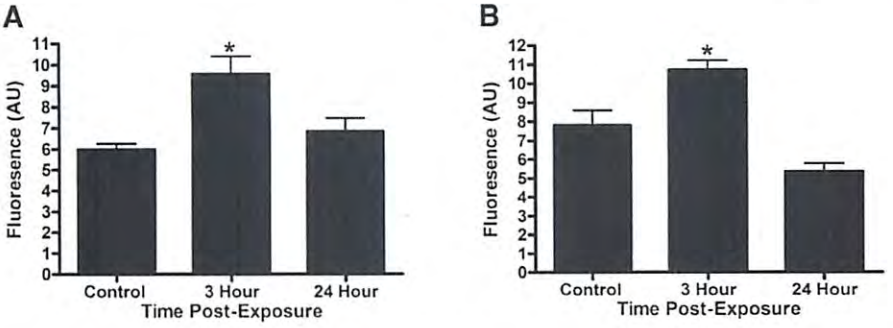

Fig. 5. Quantification of 4-HNE (**A**) and 3-NT (**B**) levels in brain sections after exposure to blast. Significant increases in 3-NT and 4-HNE levels were observed at 3 hr after exposure compared with control. At 24 hr after exposure, 3-NT and 4-HNE levels had returned to control values. The data represent the mean fluorescence ± SEM. Significance with ANOVA followed by post hoc analysis (SNK) as follows: $^*P < 0.05$.

progression of neuropathological processes that could be relevant to blast-exposed populations. The systemic effects of the level of blast (~ 120 kPa) used in the present study were previously characterized and were shown to induce moderate pulmonary damage with acute inflammatory responses that were resolved by 8 days (Chavko et al., 2006). The exposure to this level of BOP does not produce lethal damage, and the survival rate of rats at 120 kPa under isoflurane anesthesia is more than 90%. In the present study, we looked for some basic neurological and neuropathological changes in the brain and demonstrated that 120 kPa BOP exposure produced acute reflex suppression in rats that was similar to suppression observed in other models of diffuse brain injury (Denny-Brown and Russell, 1940; Dixon et al., 1987; Fijalkowski et al., 2007). This extent of reflex suppression is consistent with concussion and mild TBI, which closely resembles the clinical manifestation of bTBI (Jones et al., 2007; Bruns and Jagoda, 2009). Previous studies have demonstrated a dose-response relationship between the extent of acute neurological impairment and the overall cognitive outcome following experimental diffuse brain injury (Beaumont et al., 1999). Long and colleagues (2009) reported that, following 126 kPa BOP exposure, there is transient cognitive dysfunction; the observed reflex suppression in the present study predicts these findings by Long et al. The results also demonstrate increased IgG immunoreactivity in the brain 3 and 24 hr after exposure, indicating increased early permeability of BBB followed by microglia activation at 5 and 10 days postexposure.

BBB breakdown has been shown to occur early after TBI; it is mostly transient, and the time course of the BBB opening varies in different models. In a fluid percussion model of injury, BBB damage in the rat brain was most pronounced within the first hour after TBI and was reestablished by 6 hr after injury (Tanno et al., 1992). Others also reported that the BBB was sealed within a few hours after fluid percussion injury (Enters et al., 1992). We observed significant BBB breakdown

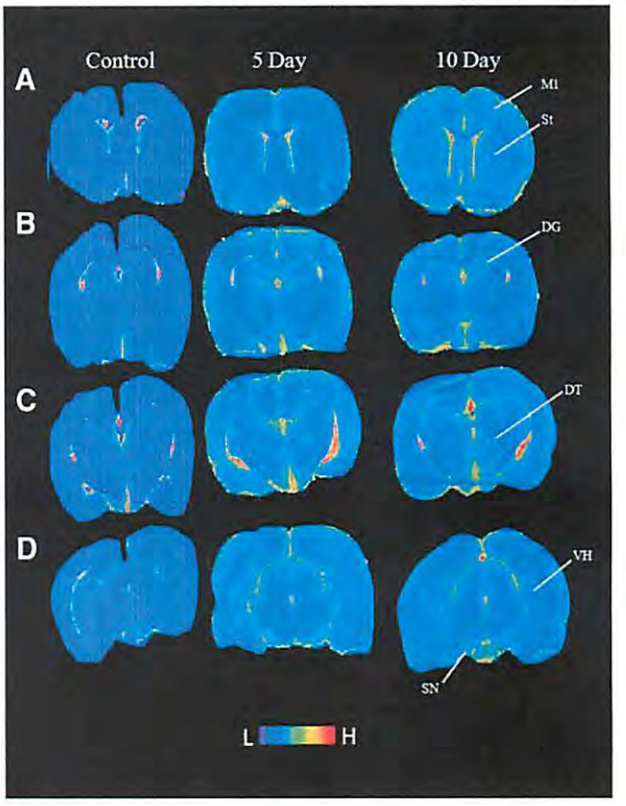

Fig. 6. Effect of exposure to 120 kPa BOP on TPSO expression. Pseudocolored autoradiogram of brain sections labeled with [^{3}H]PK11195 in control and in 5- or 10-days-postexposure animals. Four different levels are shown: anterior striatum (**A**), dorsal hippocampus (**B**), dorsolateral thalamus (**C**), and ventral hippocampus (**D**). Brain are regions abreviated as follows: primary motor cortex (M1), striatum (St), dentate gyrus (DG), dorsolateral thalamus (DT), ventral hippocampus (VH), and substantia nigra (SN). Prominent areas of high TPSO density in control animals are restricted to ventricular/choroid plexus regions. High- density areas of TPSO in exposed animals are the dentate gyrus and substantia nigra. The scale at bottom shows the range from low to high expression. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

TABLE I. Quantification of TPSO Expression in Selected Brain Regions[†]

Region	Control	5 Day		10 Day	
		Ipsilateral	Contralateral	Ipsilateral	Contralateral
Primary motor cortex	41.25 (6.8)	41.53 (5.5)	43.79 (3.6)	44.89 (1.9)	48.32 (3.0)
Striatum	38.22 (4.9)	37.29 (7.3)	38.47 (5.8)	42.68 (6.1)	43.99 (3.7)
Dentate gyrus	37.07 (5.4)	43.95 (4.4)*	45.34 (3.3)**	48.04 (3.4)*	47.68 (2.5)*
Dorsolateral thalamus	37.69 (11.0)	39.15 (6.5)	39.88 (7.2)	41.84 (3.1)	42.09 (3.0)
Substantia nigra	39.28 (8.0)	42.80 (6.0)	45.64 (3.5)	45.70 (3.5)	49.74 (3.1)**
Ventral hippocampus	40.41 (11.3)	42.06 (3.4)	43.45 (4.9)	42.97 (4.7)	48.28 (3.5)**

[†]There was a significant increase in PK11195 binding in the dentate gyrus at both survival intervals. In addition, the TPSO expression was increased in both the contralateral substantia nigra and the ventral hippocampus 10 days after blast. The data represent the mean density of [^{3}H]PK11195 binding (arbitrary optical units) ± SEM.

*$P < 0.001$ vs. control.

**$P < 0.05$ vs. control.

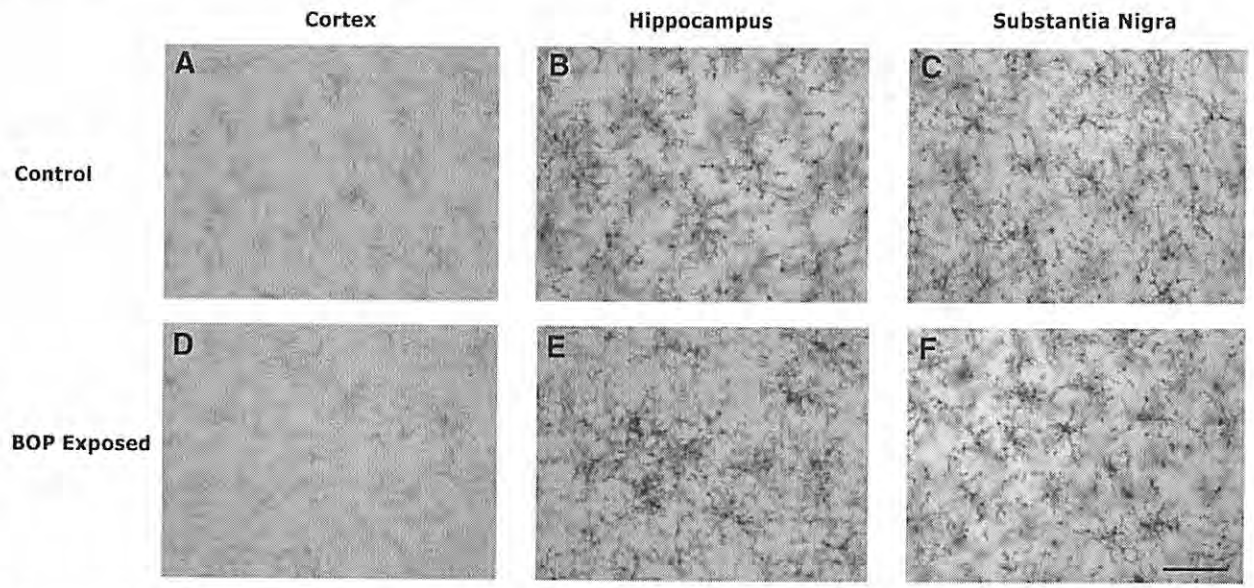

Fig. 7. Effect of 120 kPa BOP on microglia morphology in selected brain loci. To confirm PK11195 autoradiographic data demonstrating microglial activation in the dentate gyrus and substantia nigra, antibodies against CD11b/c were employed to visualize microglial morphology. In sham animals, all regions that were evaluated contained microglia with resting morphology characterized by highly ramified processes (**A–C**). In the cortex of animals exposed to blast, microglia also displayed resting morphology (**D**). Exposure to blast resulted in activated microglial morphology characterized by microglial clustering in the hippocampus (dentate gyrus; **E**). In addition, activated microglia morphology in the substantia nigra is revealed by microglia with rounded cell bodies and reduced ramifications (**F**). Scale bar = 50 μm.

following blast, which was limited to layer I of the cortex and was considerably smaller in comparison with diffuse fluid percussion brain injury, with which overt BBB breakdown was observed throughout the neocortex, hippocampus, and thalamus (Kelley et al., 2007). Similarly to other TBI models, BBB disruption was transient and appeared to have recovered by 3 days.

It is generally thought that the BBB opening is a major factor contributing to the presence of brain edema observed in TBI. However, in some TBI models, edema formation clearly does not correspond to BBB opening (Beaumont et al., 2000). Instead, a cytotoxic mechanism is thought to be the major mechanism of edema formation in TBI. Despite this, a permeable BBB can provide a route for passive fluid movement and thus worsen the cytotoxic cell edema. Recently, Saljo et al. (2009) reported a blast dose-dependent rise in ICP in rats exposed repetitively three times to blast and an increasing time delay in elevation with decreasing intensity of exposure. Although the initial elevation took place within 30 min after exposure to 60 kPa, it did not appear until after 2 hr and 6 hr after exposure at 30 and 10 kPa, respectively. The delayed BBB opening and similar time course in ICP increase may indicate a contribution of a vasogenic component to brain damage after exposure to blast.

BBB breakdown in TBI can be a result of the initial insult or the product of secondary injuries, such as accumulation of inflammatory cytokines. Whalen et al. (2000) found a pattern of transient BBB opening that

was independent of inflammation; the BBB was leaky within the first 30 min to 4 hr after controlled cortical impact in rats, whereas white blood cell accumulation peaked at 24 hr. It was concluded that BBB damage was not related to the white blood cell accumulation and inflammation. It was previously shown that exposure to BOP produced early (1–3 hr postexposure) recruitment of polymorphonuclear leukocytes (PMNs) in peripheral blood (Gorbunov et al., 2004) at the level of blast in the present study. Therefore, a contribution of inflammation to the BBB opening cannot be ruled out and requires further investigation.

In virtually all pathological conditions of the CNS, accumulation and activation of microglia precede or are concomitant with neuronal and glial cell damage. Microglia, as the principal immune cells in the CNS, are known as a source of highly cytotoxic substances, including proinflammatory cytokines, reactive oxygen intermediates, proteinases, and complement proteins (Rieske et al., 1989; McGeer et al., 1993; Giulian et al., 1995). Microglia-derived oxygen free radicals as well as subsequent reaction products, hydrogen peroxide and peroxynitrite, have the potential to harm cells and have been implicated in contributing to oxidative damage and neurodegeneration in neurological diseases. Our findings show the inflammatory response in the brain long after initial impact and with the absence of detectable cell loss. It has been suggested that microglial activation in TBI cannot be attributable exclusively to the infiltration of blood-borne macrophages or molecules from the circulating blood. This implies that the microglial activation occurring is caused largely by intrinsic mechanisms within brain tissue, e.g., as a response to diffuse axonal injury. Several recent reviews have suggested that inflammation may serve as a biomarker for bTBI (Agoston et al., 2009; Svetlov et al., 2009). After a single severe blast, Kaur and colleagues (1995) previously demonstrated that there is widespread microglial activation up to 14 days postblast. In addition, ultrastructural findings by Cernak and colleagues (2001a,b) demonstrated glial reaction in the hippocampus of rats exposed to high levels of BOP. Recently, Long and colleagues (2009) reported that, after moderate levels of BOP (126 kPa), there was widespread gliosis throughout all levels of the brain. However, it was unclear whether microglia and/or astrocytes were activated, and the regional pattern of inflammation remained uncertain.

It was recently shown that increased PK11195 binding in some brain areas corresponds with microglial activation following TBI (Benavides et al., 1990; Raghavendra Rao et al., 2000). To provide higher anatomical resolution of regional microglial activation following a moderate level of BOP, we selected PK11195 binding as a surrogate marker of microglial activation. We demonstrated increased PK11195 binding in the dentate gyrus, substantia nigra, and ventral hippocampus 5 days and 10 days after exposure to blast. At 10 days, the inflammatory response appeared to be evolving further, insofar as increased binding was observed compared with 5 days. Whereas at 5 days after exposure the distribution of lesions was localized bilaterally in both hemispheres, later at 10 days increased binding density was distributed more contralaterally to the site of impact. This distribution of microglia has some analogy with previously published data on the vasospasm distribution after exposure to blast. Unlike conventional TBI, traumatic cerebral vasospasm (TCV) often occurred in vessels far distal to the area of fragment penetration in the hemisphere contralateral to the injury. It was suggested that, in addition to the fragment injury, propagation of the blast wave through the tissue could have caused TCV (Armonda et al., 2006). Another possibility is migration of toxic components of microglia activation products from the site of injury into distant regions (Gong et al., 2000).

The results presented clearly illustrate the selective vulnerability of the hippocampus and substantia nigra to BOP exposure. The high density of NMDA receptors present in the hippocampus may be partially responsible for the vulnerability of the hippocampus to excitotoxic insults (Butler et al., 2009). It was shown by positron electron tomography (PET) that PK11195 can visualize excitotoxic lesions in the living human brain (Groom et al., 1995). High vulnerability to oxidative stress of dopaminergic neurons in the substantia nigra and resultant mitochondrial dysfunction may contribute to the observed increase in microglia activation in substantia nigra (Hastings, 2009).

Cernak and colleagues (1999) demonstrated that high levels of BOP result in an increase in brain nitric oxide production and lipid peroxidation as early as 3 hr postexposure that resolves by 5 days postexposure. However, prior to the current study, oxidative stress following a moderate level of blast remained undetermined. Here we report that as early as 3 hr postexposure there is a significant increase in 3-NT and 4-HNE levels. At 24 hr, 4-HNE and 3-NT levels had returned to control values. This indicates that there is a rapid increase in oxidative stress following moderate BOP exposure and that oxidative stress is at least partially occurring concomitantly with BBB breakdown. It has been postulated that vascular abnormalities following TBI are dependent on the production of oxygen radicals (Kontos and Povlishock, 1986; Pun et al., 2009). Therefore, antioxidants may represent one potential neuroprotective target for therapeutic intervention following bTBI, which may in turn reduce other downstream pathological processes (i.e., BBB breakdown).

In summary, our data demonstrate that bTBI likely shares some common mechanisms of injury with other TBI models. In this study, we have demonstrated that inflammation likely plays a role in the neuropathology associated with bTBI. We report significant BBB breakdown that resolved by 3 days postexposure. Oxidative damage markers increased rapidly and were resolved by 24 hr postexposure. Taken together, our results clearly show that the brain is susceptible to BOP exposure and that specific brain regions are more susceptible to blast than others.

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